Amendments to the Specification:

Please replace the paragraph beginning at line 23 on page 5 with the following amended paragraph:

The preferred molecular weight of AFPs of the invention is ~~form~~from 8 to 16 kDa, more preferred 10-14 kDa, where this molecular weight is determined from the gene sequence or by mass spectrometry of the unmodified form e.g. in unglycosylated form.

Please replace the paragraph beginning at line 27 on page 8 with the following amended paragraph:

Applicants also have found that AFPs of the above sequence have improved ice-~~recrystallidation~~recrystallisation inhibition properties. A suitable test for determining the ice recrystallisation inhibition properties is described in the examples and involves the quick freezing to -40°C follow by storage for one hour at -60°C. Preferably AFPs which are subject to this test after heat-treatment result in an ice crystal particle size which is less than 5 μm larger than the ice crystal size of a sample with the same AFP which was not heat-treated. Preferably the difference is less than 3μm, most preferred less than 1 μm.

Please replace the paragraph beginning at line 32 on page 14 through line 3 of page 15 with the following amended paragraph:

Generally this test can be applied to any suitable composition comprising AFP and water. Generally the level of AFP in such a test composition is not very critical and can for example be from 0.0001 to 0.5 wt%, more preferred 0.0005 to 0.1 wt%, most preferred 0.001 to 0.05 wt%, for example 0.01 wt%.

Please replace the paragraph beginning at line 3 on page 23 with the following amended paragraph:

The active fraction was vortexed in an equal volume of phenol : chloroform : isoamyl alcohol (1:1 [24:1]) for 1 minute. It was then centrifuged at 13,000 x g for 30 minutes. The aqueous (upper) and organic (lower) phases were collected separately, no interface was ~~obersvable~~observable. The two phases were then precipitated by the addition of ten volumes of cold acetone and held at - 20°C for 16 hours. The resulting pellets were resuspended in 50 mM tris/Cl buffer, assayed for RI activity and run on SDS PAGE.

Please replace the paragraph beginning at line 8 on page 26 with the following amended paragraph:

The Lolium cDNA was cloned into a pPIC9 vector with an α - factor signal sequence to ensure secretion from the cell and glycosylation. All enzymes were from Boehringer Mannheim and used according to the manufacturers ~~instructons~~instructions. Construction of expression vectors, transformation and growth of Pichia were all as described in the Invitrogen Pichia Expression Kit (Version B) Manual.

Please replace the paragraph beginning at line 16 on page 26 with the following amended paragraph:

The Lolium cDNA was cloned into the pPIC9 vector as a PCR amplification fragment, with compatible restriction ends for ligation into the pPIC9 vector. This was produced using Lolium cDNA as the template and the primers GTATCTCTCGAGAAAAGAGATGAGCAGCCGAACACGATT (SEQ. ID No. 7) and TTAATTCGCGGCCGCCTGTAGGAAAAGTATGGTATATC (SEQ. ID. No. 8) which introduced a Xho1 restriction site at the 5´ end and a Not1 restriction site at the 3´ end of the amplification fragment and ensured that the Lolium cDNA was in frame with the secretion signal open reading frame. The reactions were carried out in a thermal cycler using Taq DNA polymerase and Pfu proof reading enzyme (Boehringer Mannheim) for 30

cycles (1 minute at 94°C, 1 minute at 55°C and 1 minute at 72°C). All subsequent PCR reactions were carried out under the same conditions but ~~withiut~~without Pfu enzyme. The Xho1/Not1 cDNA fragment was then cloned into the Xho1/Not1 digested pPIC 9 vector and transformed into competent E Coli cells (strain XL1-blue). After transformation, they were plated onto LB plates with 50µg ml ampicillin and grown at 37°C for 16 hours. Then, 20 ampicillin resistant transformants were picked and analysed for integration of the Lolium cDNA by PCR using the 5´ AOX1 and the 3´ AOX1 primers that had been synthesised as specified in the Invitrogen Kit Manual.

Please replace the paragraph beginning at line 23 on page 27 with the following amended paragraph:

Initially the colonies were ~~innoculated~~inoculated into BMGY medium and grown at 30°C for 24 hours after which the cells were spun down and transferred to BMMY medium to induce expression and returned to 30°C for a further 24 hours. Two more additions of methanol to 0.5% were made at 24 and 48 hours to maintain induction. The cells were then removed by centrifugation and an aliquot of the medium adjusted to 30% sucrose and assayed for ice recrystallisation inhibition activity. The medium of all the transformants contained significant RI activity whereas medium produced in the same way from control Pichia without the integrated Lolium cDNA had no activity.

Please replace the paragraph beginning at line 9 on page 28 with the following amended paragraph:

E. coli XL-1 blue was used for all cloning steps; cultures were grown on 2YT medium with selection by Ampicillin or Kanamycin as appropriate. All oligonucleotide primers were ~~synthesisied~~ synthesized on a Perkin Elmer 381A DNA Synthesiser, using the phosphoramidite method as recommended by the manufacturers. Nurse culture plates were prepared by plating 2ml of a cell suspension of *Nicotiana bethiama* on a petri dish containing 25 ml of MS salts, B5 vitamins (1mg L nicotinic acid, 1 mg L pyridoxine, 10mg L

thiamine, 100 mg L inisitol) + 1 mg L 2-4D, 0.2 mg L BAP & 0.8% agar. The cells were swirled to cover the agar and then covered with a sterile filter paper disc.
Selection media = MS basal media + 3% sucrose, 0.2 mg L IAA, 1 mg L BAP, 0.9% agar + 500 mg L cefotaxime & 100 mg L kanamycin.

Please replace the paragraph beginning at line 25 on page 30 and line 6 on page 31 with the following amended paragraph:

Nicotiana tabacum (var Petit Havana) seeds were surface sterilised for 10 minutes with a solution of 10% sodium ~~hyperchlorite~~ hypochlorite and after rinsing three times with distilled water, were transferred to Murashige & Skoog basal medium + 3% sucrose + 0.9% agar. The seeds were grown for two weeks and then thinned to 2 per vessel after which sterile plantlets ~~wer~~were obtained by taking monthly shoot cuttings into MS basal medium + 3% sucrose + 0.9% agar. *Agrobacterium* cells containing the appropriate plasmid were cultured overnight in Lennox broth (5g L^{-1} NaCl, 10 g L^{-1} yeast extract, 10 g L bacto tryptone and 15g L agar), after which they were recovered by centrifuging for 10 minutes at 3000 x g and resuspended in MS basal medium + 3% sucrose. Tobacco leaf discs cut with a sterile cork borer were infected by incubating for 10 minutes with the *Agrobacterium* culture after which they were patted dry on sterile filter paper and plated face down on a nurse culture plate of tobacco cells.

Please replace the paragraph beginning at line 19 on page 31 with the following amended paragraph:

The infected leaf discs were incubated at 26°C for 2 days in a light intensity of 2000lux and then removed from the nurse culture plates to selection media. They were then incubated at 3000 lux, 26°C, 16 hours day/ 8 hours dark and transferred every two weeks to fresh media. As new growing shoots appeared at the edge of the disc they were removed to MS basal media + 3% sucrose, 500 mg L cefotaxime & 100 mg L kanamycin for rooting.

Please replace the paragraph beginning at line 32 on page 34 with the following amended paragraph:

The fusion protein was purified by metal affinity column and the thioredoxin removed by digestion with thrombin. The identity of the cleaved AFP was confirmed by ~~potein~~protein sequencing of the N-terminus and this cleaved protein was shown to also have significant ice recrystallisation inhibition activity.